SELECTION OF RACHITIC AND ANTI-RACHITIC DIETS IN THE RAT

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The experiments to be described deal with two distinct, though closely related problems. The problem first set for investigation was to determine the additional difficulty a rat on a high-calcium, low-phosphorus rachitogenic diet would undergo in order to obtain a food tending to adjust the mineral balance and prevent or alleviate rickets. The second problem, a more fundamental one, the investigation of which was indicated by the equivocal nature of the results obtained on the first, was to determine the nature of selection between the same two diets under simple choice conditions, with a view, in particular, toward investigation of the relationship between selection and deficient nutritional status of the animals concerned.

There is no work extant that deals specifically with either of these problems. The correlation between food selection and the differential motivating value of the same foods as goals in food-seeking activities of greater complexity has received little attention from students of animal behavior. Fischel (11, 12), it is true, in investigating a different problem used a method similar in the most general features to the one to be described. Pre-

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ferred foods were made more difficult of access than non-preferred foods in a choice situation. Maslow and Groshong (20) measured the differential effectiveness as rewards in a learning problem of foods which had been ranked previously on a preference scale by means of choice tests. The results of both these experiments suggest the importance of food preferences as motivating factors. At least one study of the differential incentive value of different foods in maze learning in rats (27) supports this consideration, although selection tendencies or preferences are not necessarily involved in such a situation, and the conclusions of this earlier work have been significantly questioned. The whole field is in need of thorough investigation.

No one has attempted to determine the ability of rats under conditions of rachitic feeding to select diets whose mineral content has been adjusted in a beneficial direction, as in the present experiments. Harris and co-workers (14) mention an investigation of the ability of similarly deficient animals to obtain readjustment through selection of foods containing the necessary vitamin (D), with negative results. The animal's ability to select in accordance with nutritional need has, indeed, been made the subject of a number of investigations. In general, food selection as a function of nutritional need has been a matter of interest for the biochemist and the worker in applied fields of nutritional science. Food selection as a system of overt behavior related to various other such systems has been marked out in recent years as a matter for investigation by the psychologist (30); but only certain aspects of food selection have attracted actual experimental attack on the part of psychologists. It is impossible to review here the work of either type of investigator with any degree of thoroughness. The relevant results may be briefly summarized.

(1) Animals allowed to select their own ration from a variety of natural foodstuffs compare favorably in growth and health with available norms (10, 22). No negative instances have been reported, but the investigations have not been numerous. Where artificial preparations are used instead of natural foodstuffs, the results of different experimenters have been conflicting (17).

(2) As to whether animals may avoid specific deficiencies where choice is confined to foods, one containing, one not containing the factor necessary to prevent the deficiency in question, the findings are again conflicting (22, 17).

(3) Animals experimentally depleted of specific factors do, in the case of certain of these, select to obtain them. The best-established findings are those of Harris and co-workers (14) on vitamin B deficiency.

(4) Animals tend to regulate their consumption of certain foods in accordance with energy requirements (15, 16).

(5) Selection tendencies (preferences) may occur independently of nutritional status (2, 11, 12, 26, 30). That nutritional status has been definitely ruled out in these experiments is not certain. Such selection tendencies have been observed in at least two classes and a number of species of vertebrates. They are relatively stable. That they are modifiable by a variety of factors has been established (2, 11, 12, 26, 30), but the possibilities of modification have by no means been fully explored. Whether selection tendencies definitely correlated with nutritional condition are also subject to these influences is not known.

The relationship of deficient nutritional status to other aspects of behavior than food selection has been treated in a number of studies. Maze learning in rats has been the chief point of attack. A clear-cut detrimental effect on learning has been demonstrated in the case of vitamin B deficiency, at least during the early stages of post-natal development (25). In the case of other deficiencies studied from this angle—vitamin A deficiency (21), protein deficiency (1, 3), rickets (13), to mention the most important—the evidence is either negative or conflicting. The work that has been done is of great importance, but its analysis of effects where present has not proceeded far enough to give us much insight into the precise relation of various deficiencies to the whole behavior system of the animal or even to learning as a fundamental process. What evidence we possess points to a probable effect of these deficiencies on spontaneous activity as well as maze learning (1, 3). These results in general do not furnish sufficient basis to draw conclusions regarding the underlying nature of the effects. It is impossible to trace a connection between the findings in this field and those in the field of food selection.

DIETS AND ANIMALS

The present experiments, as noted, deal only with the effect of one type of deficiency on food selection and with selection between one type of deficient diet and another diet, of a nature to remedy the deficiency. Throughout these experiments three diets were used. Of these only two were used in selection tests—a rachitic and an anti-rachitic diet. The former was Steenbock's rachitogenic diet number 2965 (28); the latter, a modification of the Steenbock diet suggested by Dr. E. A. Park of the Harriet Lane Home of the Johns Hopkins Hospital. For some of the experiments the diets were prepared by the Harriet Lane laboratory; in most, they were mixed in our own laboratory, with strict adherence to the technique prescribed. It is possible to compare the effects of different lots of diet, but no differences in selection ascribable to this factor were observed.

The formula for the Steenbock diet is as follows:

	per cent
Ground yellow corn	76
Wheat gluten	20
$CaCO_3$	3
NaCl	1

The formula for the modified anti-rachitic Steenbock is as follows:

	per cent
Ground yellow corn and wheat gluten in ratio 76:20	94.25
$CaCO_3$	3.0
NaCl	1.0
Na_2HPO_4	1.75

In several of the experiments a basic mixture of corn, gluten, calcium carbonate, and sodium chloride in correct proportions was first prepared and the two diets obtained by adding to a definite quantity of this mixture the proper amount of corn and gluten in the case of the rachitic diet and di-sodium phosphate in the case of the anti-rachitic diet. In this way two lots of diet could be offered for selection which varied only in respect to the critical nutritive factors. Slight differences in ingredients and preparation could thus be reduced to a minimum. The change to this procedure had no apparent effect on the results.

Although it is not essential to include a survey of the work on experimentally produced rickets in rats, it is necessary to state briefly the experimental basis for the use of these diets in the investigation of nutrition problems. The diets used have been well standardized in the biochemical field. It is now well established in general that a severe type of rickets can be produced in young rats, in the absence of vitamin factors obtainable from diet or from certain types of radiations, by diets which contain a high ratio of calcium to phosphorus within certain limits of absolute amount of these elements (6, 23). In recent years one of the most widely used of such diets has been the rachitic diet of the present experiments. It is now generally conceded, also, that rickets produced by this type of diet can be prevented or healed merely by the addition of a phosphate in amounts sufficient to lower the calcium:phosphorus ratio a certain degree (19). The particular anti-rachitic diet used in the present experiments has a calcium:phosphorus ratio of approximately 1:0.5 in contrast to the 5:1 ratio of the rachitic diet. It has been found to prevent rickets when mixed in equal amounts with the latter (24).

The third diet used as an experimental ration was the Steenbock-Bills stock diet (4), which has been widely employed in the maintenance of laboratory rats and is generally conceded to be adequate for growth. Its formula is:

	parts
Ground yellow corn	76
Whole milk powder	33
Linseed oil meal	16
Crude casein	5
Alfalfa meal	2
$CaCO_3$	0.5
NaCl	0.5

For the two experiments in which this diet was used, it was prepared according to the exact formula. Stock rats at all times and experimental rats, for the period prior to the regime of experimental feeding, were fed a modified form of this diet. For this, corn, linseed oil meal and alfalfa were measured by volume instead of weight, for convenience in the preparation of large quantities of food. Occasional supplements of lettuce were pro-

vided the stock at all times and experimental animals prior to the start of experimental feeding.

In general, elaborate precautions were used in the preparation of experimental diets. All ingredients were weighed on a sensitive balance to the nearest 0.1 gram. All mixtures were made up in 1000-gram lots and thoroughly mixed by hand. Containers were cleaned before each mixing and weighing. Diets were kept in separate, tightly-closed jars, from which the ration for each day's experiment was directly transferred to food cups.

The animals used were all of the albino stock bred in the laboratory for a number of years, originally having been obtained from the Wistar Institute. This stock has been maintained under conditions of average adequacy for health and normal development. Growth records of breeding animals taken during the period of most rapid growth compare favorably with Donaldson's (7) norms. Normal, healthy-appearing litters only were selected for use in the experiments. It was the general practice to use a number of whole litters for a particular experiment. The animals were placed on the experimental regime a few days after being separated from their mothers, which occurred at 21 to 25 days of age. During the intervening period they were maintained on the regular ad libitum stock feeding, individuals in the meantime having been marked and sexes segregated. After the beginning of experimental rationing the groups were kept in large stock cages, with sexes separate, and under conditions similar to those of the stock rats, except that feeding was definitely controlled by the methods to be described.

MAZE EXPERIMENTS—APPARATUS AND PROCEDURE

The problem originally set up, as noted above, was to determine the additional difficulty rats fed on the rachitic diet would undergo in order to obtain the anti-rachitic diet. It was decided, as a first step, to test the animals in a simple choice maze, consisting essentially of a long path and a short path leading to separate food boxes, with anti-rachitic diet at the end of the long path and rachitic diet at the end of the short path. It was

necessary, however, first to test selection between the paths with the food reward at the end of both the same. If the short path were selected consistently under these conditions, it could be concluded that the long path was of a genuinely greater difficulty for the animals and that selection of the long path, with a different food reward at its end, could be regarded with other conditions properly controlled, as a function of the change in food offered. Thus the first maze situation constituted a test of the

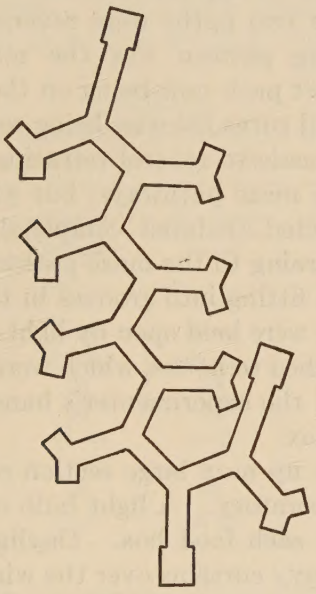


FIG. 1

validity of the apparatus as a means of attack on the problem set up.

The apparatus was constructed of the standard Y-units described by Warner and Warden (29). These units can, of course, be put together in a large variety of maze patterns. The pattern adopted for use is illustrated in figure 1. It consists of an entrance box, a straightaway of one section, and two paths, each leading to its own food box. The actual ratio of the distance

(entrance point to food box) of the long path to that of the short path was approximately 4:1. The relative difficulty of the two paths involves, of course, in addition to distance, the greater complexity of turns in the long path. It may be noted from the figure that blinds are provided at all junctions except the choice point. In all the experiments except a special investigation of one group of animals, to be mentioned, these blinds were completely blocked off by sheets of galvanized iron covering the openings of each. In the experiments on four groups the side-to-side positions of the two paths were reversed for one series of trials. The resulting pattern was the mirror image of the original one, the short path now being on the left and the right-left relationship of all turns likewise being reversed.

No attempt was made to control retracing on the part of the animals while in the maze pathways; but when a food box had been definitely selected (entered completely) the animal was prevented from returning to the maze pathway by gently lowering one of the doors fitting into grooves in the sides of the food boxes. These doors were held open by light cords running along the ceiling and attached to hooks, which were fastened at a point within easy reach of the experimenter's hand in his position behind the entrance box.

The maze was set up on a large section of empty floor space in a room of the laboratory. A light bulb of the same wattage was suspended over each food box. Daylight was shut out as far as possible by heavy curtains over the windows. The experiments were conducted under conditions of extreme quiet, no disturbing influence in the room being permitted while they were in progress. The floor under the maze was cleaned frequently. Food was available in shallow troughs in each feeding box, a fresh supply being placed in the troughs daily. A water bottle was attached to the inner wall of the feeding boxes.

Eight groups of animals were tested in these experiments, a total of forty-nine finishing the entire routine. The animals were put on the rachitic diet at 24 to 30 days of age. The variations in age at the start of rachitic feeding and at the start of the maze tests of actual diet selection are shown in table 1.

Groups I, II, and III were given a period of ad libitum feeding on the rachitic diet in their living cages to accustom them to the diet. This period lasted 7 days in the case of groups I and II; 1 day, in the case of group III. After this these animals were given an orientation period in the maze; the other groups were given this at the start of experimental feeding. During this period the group as a whole was allowed to explore the maze freely for several hours each day with location of food and other features of the maze situation exactly the same as in the individual tests that followed. The initial orientation periods were continued for several days (2 to 6) on all the groups. The pur-

TABLE 1

Critical age variations for each group of animals in the maze experiments, with relation to final results

GROUP	NUMBER	AGE AT START OF RACHITIC FEEDING	AGE AT START OF SELECTION TESTS	LENGTH OF RACHITIC FEEDING AT START OF SELECTION	RESULTS
		days	*days*	*days*	
I	3	28–31	37–40	9	Positive
II	6	28–31	52–55	24	Negative
III	9	24	38	14	Positive
IV	4	30	65	35	Negative
V	4	26–30	61–65	35	Negative
VI	7	30	65	35	Negative
VII	6	26	61	35	Negative
VIII	10	28–30	36–38	8	Negative

pose was to accustom the animals thoroughly to the maze situation before the actual choice tests were made.

The first experimental situation, designated hereafter as maze situation 1, was designed to determine whether rats would show a consistent tendency to take the short path when the same kind of diet was available in both feeding boxes. Rachitic diet was used for these tests. Each animal of groups I, II, III, VIII was given 5 trials the first day (groups IV, V, VI, VII, 5 trials a day for 5 days) and thereafter 10 trials a day. Individuals of a group were run in chance order. In each trial a rat was placed in the entrance box of the maze and allowed to run. As soon as it completely

entered a food box the door was closed, and the animal was retained in the food box a sufficient length of time to allow it to eat a small quantity of food. A constant time of 1 minute (except in the very earliest trials) was adopted for groups I, II, III. One-half minute was used for the later groups. When the determined time had elapsed the animal was removed from the box and placed in the entrance box for the next trial. At the completion of the daily series of trials it was placed in an individual feeding box. The total length of daily feeding was restricted to $\frac{1}{2}$ hour for groups I, II, III; to 3 hours, for the later groups. When most of the rats in a group had achieved the standard of an average selection of the short path for 70 per cent of the daily series—individual records only were considered—the tendency to select the short path was considered to be established sufficiently for transfer to the crucial maze situation involving actual dietary selection. In four groups (IV, V, VI, VII) a more thorough investigation of the ability to select the short path was undertaken. These animals were given an additional 30 trials with the right-left relationships of the maze reversed, as previously described, and following this, 20 trials with the original maze pattern.

A second situation was set up at this point for each group to determine whether the animals would alter their preference for the short path in order to obtain a more adequate diet. This situation will be designated maze situation 2. All conditions were the same for each group except that the anti-rachitic diet was now placed at the end of the long path. The rachitic diet, as before, was present at the end of the short path. As before, an orientation period was allowed to accustom the animals to the new situation. Individual tests were then carried on according to the plan followed for maze situation 1. Failure to exhibit any modification of the short path habit after 50 to 70 trials was generally considered to indicate negative results and the experiments were abandoned at this point in groups showing this type of performance. Groups giving positive results were tested for some time to make sure of the stability of the performance, and certain changes which will be described below were introduced

into the situation to find out whether the animals would continue to run the long path under conditions of still greater difficulty.

Results. It is evident, as Dunlap has pointed out (9), that the consistency of individual performance—the critical point for investigations of preference—is not revealed by the group treatment of data sometimes used. It was thought best for the purposes of this study to evaluate group performance in terms of frequencies of individuals reaching and falling below a definite standard of performance. This standard is adjusted to permit a fairly conclusive judgment of the nature of the performance in relation to the experimental problem as stated. The same

TABLE 2

Number and percentage of animals in each group and all groups combined selecting the short path according to the standard of 40 consecutive runs with 70 per cent or over short path selections

GROUP	NUMBER	NUMBER SELECTING SHORT PATH	PER CENT SELECTING SHORT PATH
I	3	3	100
II	6	3	50
III	9	7	78
IV	4	4	100
V	4	4	100
VI	7	7	100
VII	6	5	83
VIII	10	7	70
All.....	49	40	82

general plan will be followed in the presentation of results obtained from the experiments on the second problem. Tables 2 and 3 give the records for the separate groups and for the combined groups in the two maze situations. The tabulated results do not include the data on any of the 7 animals that failed to finish the experiments. All of these animals died in the early stages of the procedure, so that their records are of no particular significance.

The results in maze situation 1 are quite clear-cut. A striking tendency to select the short path when the food rewards at the end of both paths are the same is evident. The standard adopted

as evidence of this tendency is 70 per cent or higher short path selections in 40 consecutive runs. The frequency data for the separate groups and for the combined groups are presented in table 2. Forty out of 49 rats, or 82 per cent, reached or passed this standard. It must be remembered that the standard used represents only an arbitrary minimum. The individual records, which cannot be included for reasons of space, show that many animals reached and maintained a level of performance above the standard. The selection of the short path on the part of most animals is, as a matter of fact, to be expected from other experi-

TABLE 3

Number and percentage of animals in each group and all groups combined selecting the long path in situation 2, according to the standard of 70 consecutive runs, with 80 per cent or over long path selections, including only animals that selected the short path in situation 1

GROUP	NUMBER SELECTING SHORT PATH ORIGINALLY	NUMBER SELECTING LONG PATH IN SITUATION 2	PER CENT
I	3	3	100
II	3	0	0
III	7	7	100
IV	4	0	0
V	4	0	0
VI	7	0	0
VII	5	0	0
VIII	7	1	14
All.....	40	11	28

ments, such as that of Kuo (18), in which rats have been faced with similar possibilities of selection. Individual variation from the group tendency is not important from the standpoint of the present problem, since only those animals definitely selecting the short path in situation 1 are considered in the evaluation of selection occurring in the second situation.

The results of the control test on groups IV, V, VI, VII indicated rather definitely that the animals were selecting on the basis of length of path in maze situation 1, since reversal of the maze and subsequent retest on the original situation did not

greatly upset performance. Of the 21 rats composing these four groups 19, or 90 per cent, maintained at least 70 per cent selection of the short path during 30 trials with the reversed maze pattern, and 20, or 95 per cent, maintained the same level or higher for the 20 trials of retesting on the original pattern.

The critical part of the experiment is maze situation 2. A more stringent standard—70 runs of which 80 per cent or over were long path selection—was adopted in order to rule out any ambiguity that might be relevant in the new situation. The results for each group and for the combined groups are given in table 3. Only 11 out of 40, or 28 per cent, changed to the long path according to the standard of consistency imposed. The change appears to be an all-or-none phenomenon, as it would be possible to show from the individual records. Individuals either shift quickly to predominant selection of the long path and maintain a high frequency of long path runs (90 to 100 per cent in most cases), or else continue selecting the short path to the same high degree of consistency as in the original situation.

The persistent nature of the tendency to select the antirachitic diet was demonstrated by further experiments on 8 of the 11 animals giving positive results (groups I and III). The three animals of group I were given 80 consecutive trials with a pan containing approximately three-fourths of an inch of cold water fitted tightly into the first two sections of the long path. It was necessary for the animals to wade or hop through the water, since the length of the pan and the angle in it prevented jumping over. The selections of two individuals were undisturbed. The third showed a tendency to select the short path at first but returned to the long path habit after 20 trials. Five of the rats of group III were tested on the same obstruction set-up for 10 trials without showing any disturbance. The animals of group I showed no disturbance of the long path preference when the blind alleys leading out of that path were left open by removal of the barriers, although a few errors were made on the early trials. An electrified grid occupying the same position as had the water pan—namely, the first two sections of the long path—was found to have a deterrent effect on both groups I and III with an

estimated current (grid shorted) of 6 microamperes or above. The electrical set-up was essentially similar to that described by Dunlap (8).

The results obtained from the maze experiments as a whole present two features which are of significance for the original problem. These are:

(1) That only a small proportion of the rats tested gave positive results, that is, selected the anti-rachitic diet under the conditions set up.

(2) That the selections of the rats giving positive results appeared to be of a very stable nature and persisted in spite of unusual obstructions.

It will be noted from table 3 that the animals giving positive results are, for the most part, confined to two groups (I and III) and that all the animals in these groups fall into the positive class. Some variation in treatment of different groups might fall under suspicion as responsible for the differences in behavior shown. There is, however, no consistent relationship of positive or negative results to factors in which variations between the groups existed, with one exception. Sex, age, weight at start, preliminary feeding conditions, motivation were all considered from this standpoint. Only the length of rachitic feeding prior to selection tests is of possible importance, since groups whose rachitic feeding was prolonged to 24 days or more gave negative results without exception (table 1). It must be noted that groups with shorter rachitic feeding gave negative results in some instances also.

The question of whether the animals were selecting on the basis of the anti-rachitic properties of the better diet was, of course, left open by these experiments. Their results definitely indicated the need of a more fundamental investigation of the behavior of rats in selecting between these two diets.

SIMPLE CHOICE EXPERIMENTS—APPARATUS AND PROCEDURE

The problem set for investigation in the later series of experiments was to determine the nature of selection between the rachitic and anti-rachitic diet, with a view, in particular, toward investigation of the relationship between selection and nutri-

tional status. The course of procedure and temporal arrangement of tests and feeding conditions were designed to permit comparison between results of these experiments and those obtained from the maze experiments. Animals, with maintenance feeding controlled as in the earlier experiments, were given a brief selection test with the critical diets daily after a period of deprivation. The essential modification of the situation was the presentation of the choice foods under conditions of equal and maximally easy accessibility. Further variations were introduced in the conditions of maintenance feeding of the later simple choice experiments in order to investigate the relationship of nutritional status to the observed selection trends. The dietary situation was varied in the following ways:

Experiment 1. Rachitic diet alone was given as the maintenance diet.

Experiment 2. Anti-rachitic diet alone was given as the maintenance diet.

Experiment 3. The Steenbock-Bills stock diet alone was given as the maintenance diet.

Experiment 4. Rachitic diet alone was given as the maintenance diet, but selection tests were begun only after the animals had been subjected to a prolonged period of rachitic feeding.

The first experiment was designed to determine what degree of preference, if any, for the anti-rachitic diet would appear in the simple choice situation as compared with the maze situation and to describe the course of such a preference, in case it were present. The second, third, and fourth experiments were designed to investigate the causes of selection tendencies exhibited in the maze experiments and the first of the simple choice experiments. Selection of the anti-rachitic diet in large proportion when animals are subsisting largely on this food (experiment 2) would make it appear that its selection under the original conditions was not necessarily a function of nutritional need. If the same behavior takes place when the animals are subsisting largely on normal diet (experiment 3), the existence of another factor responsible for the selections would be established. Failure to select the anti-rachitic diet under the conditions of the

second and third experiments would, on the other hand, quite definitely show that the preferences observed in the earlier work were in some way peculiar to the original feeding situation. The fourth experiment was designed as a crucial test of the hypothesis that the animals were selecting on the basis of nutritional need. If this hypothesis were true, it would be expected that preference

TABLE 4

Critical age variations (in days) for individual groups of each simple choice experiment

GROUP	NUMBER	AGE AT START OF EXPERIMENTAL FEEDING	AGE AT START OF SELECTION TESTS	LENGTH OF RACHITIC FEEDING PRIOR TO SELECTION TESTS
IX	7	23	27	4
X	8	22–23	25–26	3
XI	10	21	25	4
Experiment 1, all rats	25	21–23	25–27	3–4
XII	8	26	29	3
XIII	9	24	28	4
Experiment 2, all rats	17	24–26	28–29	3–4
XIV	11	22	27	5
XV	11	21	25	4
Experiment 3, all rats	22	21–22	25–27	4–5
XVI	8	24	53	29
XVII	6	28	56	28
XVIII	4	24	54	30
XIX	6	24	53	29
XX	3	24	50	26
Experiment 4, all rats	27	24–28	53–56	26–30

for the anti-rachitic diet would be most strikingly demonstrated after prolonged rachitic feeding.

In all these experiments, as in the maze experiments, the rats were placed on the experimental (maintenance) diet a few days after weaning, the latter taking place at 21 to 25 days of age. As before, a number of groups were tested consecutively. The ages of all individuals and groups are given in table 4. After weaning, the animals were allowed the regular stock diet ad libitum for a

day or two and then shifted to the experimental diet for 3 hours daily. A short interval between the start of experimental feeding and the start of selection tests varied slightly for the different groups, as indicated in table 4. During this interval the animals were kept and fed in the regular living cages in groups of five or six, sexes separate. At the close of this interval the animals were tested individually each day for a period of 25 to 30 days to determine the trend of selection for the critical diets. The duration of the experimental series for the various groups was: experiment 1, groups IX, X, and XI, 29, 26, and 31 days respectively; experiment 2, groups XII and XIII, 31 and 29 days respectively; experiment 3, groups XIV and XV, 39 and 34 days respectively. In experiment 4, 27 rats were tested under conditions involving further precautions than had been used in the earlier work. The diets were prepared by the Harriet Lane staff throughout the experiment. The animals were kept in living cages screened to shut out light and given only distilled water. The selection tests were continued only 14 days on account of the poor condition of the animals.

The individual selection tests were made in small cages of uniform structure. Each cage was used by only 1 animal of a group for a series of choice tests. In two experiments in which the same set of cages were used on the same days by different groups (experiments 3 and 4) three thicknesses of clean paper were placed over the bottom of the cages before each choice test in order to eliminate the possible influence of trailing. Selection cages were never used as living quarters; rats were kept in them only during selection tests and, in the case of all groups except those in experiment 3, during the 3-hour maintenance feeding period. A separate pair of food cups readily accessible from the floor and secured at opposite sides of the cage were used for all selection tests of an individual rat. To minimize spilling, these cups, of cylindrical shape, were provided with circular tops sloping toward a small central aperture designed to admit the head of the rat. A third cup was used for each rat during the maintenance feeding period when the latter was given individually. Water was available in each cage during all feeding periods.

The selection tests took place just prior to the maintenance feeding period of 3 hours and occupied 10 minutes for each animal. The animal was placed in the cage and allowed to feed freely and taken out at the end of this period, measured by a stop watch. Although the schedule of 1 choice test per day was maintained for the most part throughout the experimental series, the selection tests were omitted on a few days for each of the groups. The selection tests and maintenance feeding took place at a regular time each day. The animals were tested consecutively and in the same order; after all the animals in a group had been tested the food cups were changed and the animals returned to their respective cages for the maintenance feeding period.

Since the animals of the first three experiments were given their selection tests consecutively rather than simultaneously, the duration of food deprivation varied for each animal of a single group. The rats that were tested earlier in the day had a shorter period of time to wait between the end of each long feeding and the selection test of the next day in comparison with those that were tested later. The former animals also had to wait longer between their choice tests and the long feeding of the same day. Motivational condition thus varied somewhat from rat to rat in a particular group; but since the same order of testing was observed at all times, the motivational status of one rat relative to that of others in the group did not change. No evidence of a variation in selection produced by this factor is observable in the results. The problem of equating motivation for different individuals of the same group even in cases where the food given is inadequate in respect to quantity only is rather complex. Several elaborate techniques have been devised, but the simple plan used here of attempting to equate motivation by maintaining a constant length of feeding time and a constant interval of deprivation seems justifiable from recent experiments of Bousfield (5). Feeding behavior in terms of amount of food consumed shows a minimum of variation by the use of this method. The comparison of selection which has been undertaken here in individuals of different though controlled and constant motivational status seems justified because of the lack

of relationship between this factor and selection tendencies. These considerations do not apply to experiment 4. In that experiment the animals were tested simultaneously with the individual cages screened to obviate social influences.

Extraneous factors that might influence selection were controlled as far as possible. The tests were carried on in a room free from disturbance of any kind. The shelves on which the choice cages were placed were screened off from the room during the tests on all groups except those (IX and X) on which supplementary observational records were taken. Fresh food was weighed out for each test and the same quantity of each kind of food (an amount well over the maximal total consumption for the test period, but small enough to discourage spilling) was placed in the proper cup. In all experiments the relative positions of the diets in the cage were reversed daily. The position of the cups used for the maintenance feeding in the same cages was systematically varied to control any possible influence from this source on selection trends. Cages were cleaned at regular intervals and at the end of a series of tests on a group of animals.

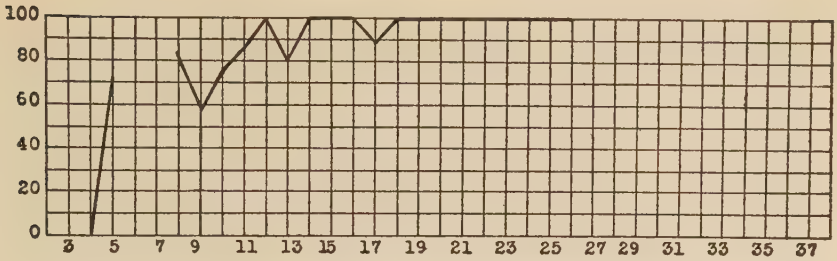
The amount of each food consumed during the choice period was measured by weighing the cups before and after feeding on a sensitive balance to the nearest 0.1 gram. The daily ratios of amount of each food consumed to total consumed during the period were used as the basis for the determination of selection trends. This method of determining preferential behavior was checked in two groups (IX and X, experiment 1) by observation of each animal's behavior during the test period. The observational recording consisted of noting by means of symbols placed in the appropriate squares of graph paper prepared for the purpose the kind of food selected (if any; if none, this fact also) during each $\frac{1}{4}$ minute of the 10-minute period, the time being followed by means of a stop watch. This check on the weighing was later abandoned as superfluous because of the close agreement between the measures of relative selection obtained from computing the time spent at each food cup and those obtained from weighing the amount of each food consumed.

Some possibility of error occurred in the food consumption

measurements obtained from the first few tests of animals maintained on the rachitic diet. These small animals ate very little at first when in the choice situation. In the summary of results, for this reason, no records taken before the animals had been on the experimental regime at least 3 days are considered. The effect on the results of the error of the scale is thus made insignificant. By this time the animals were consuming at least 0.4 gram during the choice period. By the time the third 5-day period on the rachitic diet had been reached, the amount consumed during the choice period ranged from 0.7 to 1.3 grams for animals of experiment 1. Records of total food consumption for the day were also taken occasionally on the three groups in experiment 1 and regularly in all later experiments. Animals of all groups were weighed to the nearest gram at certain stages of the experimental series; those in all experiments after the first were weighed daily.

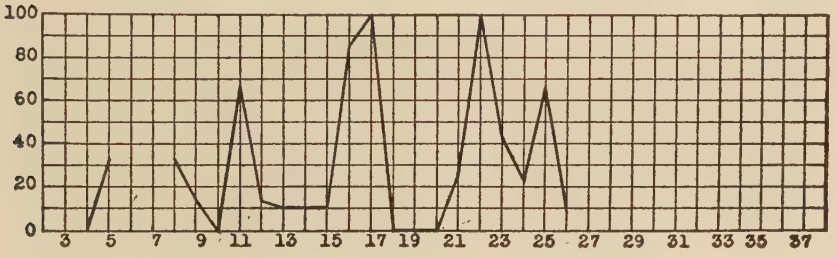
Results. For an adequate understanding of the comparative group results in these experiments, it is necessary to consider individual performances somewhat in detail. Sample curves of selection for experiments 1 to 3 are presented in graphs 1 to 6. In these graphs the daily percentage of anti-rachitic diet selected during choice periods is plotted against time. Before attempting a quantitative group comparison it is well to consider certain aspects of these data for each of the experiments.

In experiment 1, two types of individual performance can be observed. In one, such as that of rat 2, group IX (graph 1) the animal through several days of rather variable performance approaches a high level of selection of the anti-rachitic diet, reaches and maintains that level throughout the remainder of the experiment. The other type of performance, which is characteristic of a smaller number of animals—rat 3, group IX (graph 2) is an example—is one of extreme variability. Consistently high levels of selection of either diet are maintained for several days at a time, but the preference then changes to the other diet or fluctuates about the midpoint of the scale. It seems impossible to say that an animal exhibiting this sort of behavior prefers either of the diets. Practically all the animals in the first experi-



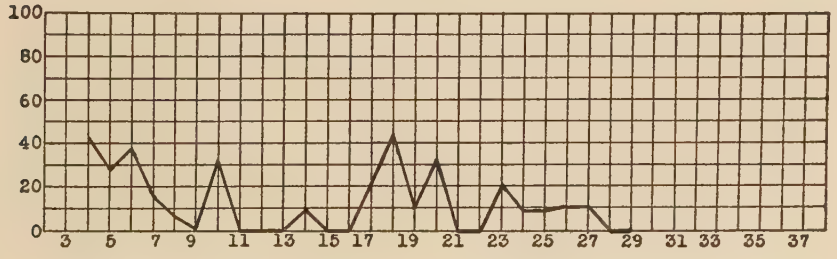
GRAPH 1. RECORD OF RAT 2, GROUP IX

Ordinates measure per cent of anti-rachitic diet selected during choice test; abscissas, days on experimental feeding.



GRAPH 2. RECORD OF RAT 3, GROUP IX

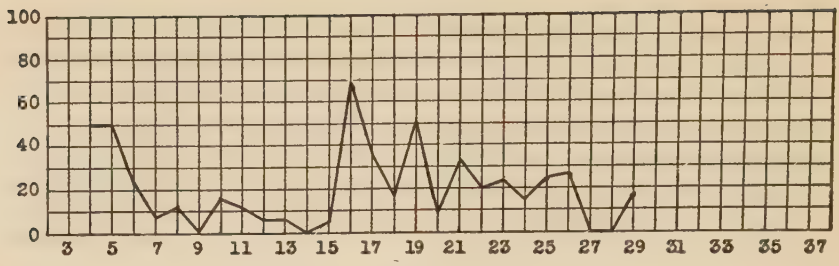
Ordinates measure per cent of anti-rachitic diet selected during choice test; abscissas, days on experimental feeding.



GRAPH 3. RECORD OF RAT 3, GROUP XIII

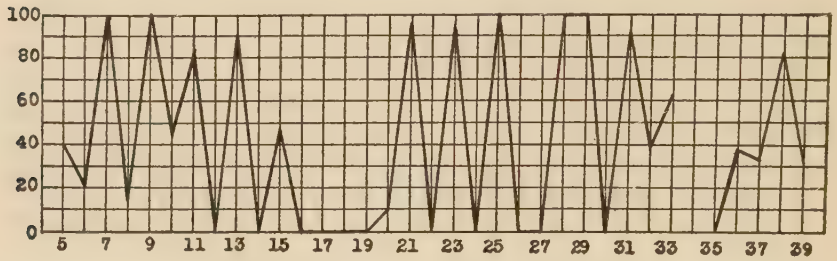
Ordinates measure per cent of anti-rachitic diet selected during choice test; abscissas, days on experimental feeding.

ment fall into one or the other of these types. There are many variations in detail between individuals; for example, some of the individuals eventually showing strong preference for the anti-



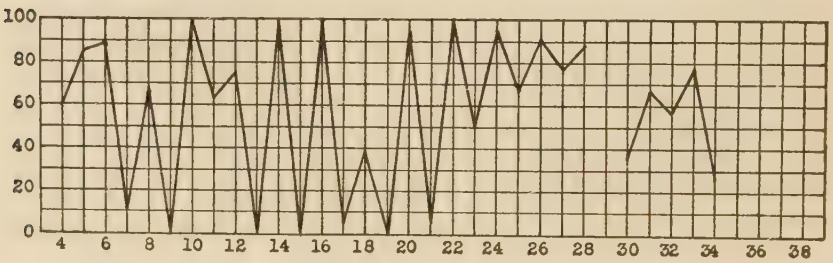
GRAPH 4. RECORD OF RAT 5, GROUP XIII

Ordinates measure per cent of anti-rachitic diet selected during choice test; abscissas, days on experimental feeding.



GRAPH 5. RECORD OF RAT 8, GROUP XIV

Ordinates measure per cent of anti-rachitic diet selected during choice test; abscissas, days on experimental feeding.



GRAPH 6. RECORD OF RAT 3, GROUP XV

Ordinates measure per cent of anti-rachitic diet selected during choice test; abscissas, days on experimental feeding.

rachitic diet develop this preference more slowly than others. But the general distinction of two types is evident in all the groups.

In experiment 2, classification of individual performances into two types may be made, although the differentiation between the two is not so sharp. One type selects the rachitic diet rather consistently; the other is variable. The graphs (3 and 4) for animals 3 and 5, group XIII, may be regarded as fairly typical. It must be noted that the consistent type of performance is not characterized by so strong a degree of preference as is shown for the anti-rachitic diet by animals of the corresponding type in experiment 1 and that the variable animals reach the higher extremes (selection of the anti-rachitic diet) only rarely.

The animals on experiment 3 do not, in general, show results permitting of classification into types. Their results are variable, with a tendency to alternate rapidly from one extreme to the other or to maintain a level close to the midpoint of the scale for long periods of time. Only one animal chose the anti-rachitic diet in consistently high proportions over the last 10 days of the experiment. Typical records are presented in graphs 5 and 6.

In experiment 4 none of the animals over the 10 to 14 day period of testing showed a consistent preference for the anti-rachitic diet. A number consistently selected the rachitic diet in large proportions; the majority were variable, with a slight tendency to go above the medium levels.

The results as described already point the way to certain conclusions, but it is rather essential for valid comparison between the groups to have quantitative measures which shall summarize the trends in performance for each group. For this purpose the experimental series for each experiment was divided into four 5-day sub-periods. The percentage consumption of anti-rachitic diet during choice tests was computed for each of these sub-periods for each individual. The next step was to tabulate the frequency distribution on a percentage scale for each group of these measures of individual preference. The results for the fourth experiment are not treated by this method because they would not be directly comparable for these measures. Table 5 presents the distributions for each of the sub-periods successively. Frequencies are expressed in percentages of group totals for purposes of easy comparison. From this table the trends of

preference for each of the groups is evident. The most important features are: First, that the animals in experiment 1 pass in the course of the four periods from a widely scattered distribution to one with the heaviest concentration at the high end of the scale, showing preference for the anti-rachitic diet to be preponderant. Second, that the animals in experiment 2 distribute themselves chiefly at the lower end of the scale showing a similar distribution through all four periods. Third, that the animals in experiment 3 show a heavy concentration at the two middle

TABLE 5

Distribution of each experimental group (in terms of per cents of the total number of individuals in the group) according to the percentage consumption of anti-rachitic diet during each of the first four 5-day periods of rachitic feeding

EXPERIMENT	PERIOD	PERCENTAGE SELECTION OF ANTI-RACHITIC DIET			
		0–24	25–49	50–74	75–100
1	1	8	36	20	36
	2	8	16	20	56
	3	16	12	4	68
	4	4	12	4	80
2	1	71	29	0	0
	2	82	6	12	0
	3	82	18	0	0
	4	82	18	0	0
3	1	5	59	27	9
	2	18	27	50	5
	3	23	36	36	5
	4	14	23	45	18

levels with tapering of the distribution into the extremes, maintaining practically the same picture throughout the course of the series.

These data show clear differences between the trends of the three experimental groups considered but do not give group measures of preference for the different kinds of diet that are convenient for a comparison of the different experiments, nor do they take into account individual records throughout each experimental series. For this purpose it is necessary to set up,

as was done for the maze experiments, an arbitrary standard of preference for an individual animal, summarizing the individual's whole performance in terms of success or failure in meeting this standard and measure group preference in terms of the proportion of each group reaching and failing to reach the standard. A satisfactory standard of this kind for the purpose of these experiments must require a rather high level of percentage selection, consistently maintained over a period of time. Animals that show a high degree of preference for a while and then fall to a lower level in later stages of the experiment must be

TABLE 6

Number of animals in each of the first three experiments reaching the standard of preference of 80 per cent average selection of a particular diet for all periods after the second, with no single period falling below 75 per cent

GROUP	ANTI-RACHITIC	RACHITIC	AMBIGUOUS	NUMBER
IX	6	0	1	7
X	7	0	1	8
XI	4	1	5	10
All rats, experiment 1	17	1	7	25
XII	0	6	2	8
XIII	0	6	3	9
All rats, experiment 2	0	12	5	17
XIV	0	1	10	11
XV	0	0	11	11
All rats, experiment 3	0	1	20	22

excluded. The standard selected as most satisfactory in the light of these considerations requires an average selection of 80 per cent for all periods of the series following the second and further that no single period within this time shall show a percentage selection of less than 75 per cent. The reason for the stringency of the standard is that one could not possibly support the assertion that the animals were selecting on the basis of their nutritive need under the conditions of these experiments unless the better diet were selected almost exclusively. It is a question whether the standard could be set too high for the purpose of this analysis.

Applying this standard to the data of all the simple choice experiments permits a differentiation of animals for each experiment into three groups; those showing preference for the anti-rachitic diet, those showing preference for the rachitic diet and those showing equivocal performance. These distributions for experimental groups 1, 2, and 3 and the included sub-groups are given in table 6. The distribution for experimental group 4 and sub-groups is given in table 7; for the animals in this experiment

TABLE 7

Number of animals in experiment 4 reaching the standard of preference of 80 per cent average selection of a particular diet for the two periods tested, with no period falling below 75 per cent

GROUP	ANTI-RACHITIC	RACHITIC	AMBIGUOUS	NUMBER
XVI	0	6	2	8
XVII	0	0	6	6
XVIII	0	2	2	4
XIX	0	0	6	6
XX	0	0	3	3
All rats, experiment 4	0	8	19	27

TABLE 8

Proportion of each experimental group showing preference

EXPERIMENT	ANTI-RACHITIC	RACHITIC	AMBIGUOUS
1	68	4	28
2	0	71	29
3	0	5	95
4	0	30	70

the standard of an average selection of 80 per cent with no measures falling below 75 per cent was applied to the data of the two 5-day periods during which these animals were tested. It will be noted that there is some variability within each of the sub-groups for all the experiments, a phenomenon which did not appear in the maze experiments.

The three experimental groups are clearly differentiated, as better shown in table 8, where the number falling into each class

is expressed in terms of percentages of group totals. Sixty-eight per cent of the 25 animals in experiment 1 show preference for the anti-rachitic diet. Seventy-one per cent of the 17 animals in experiment 2 show preference for the rachitic diet. About a fourth of each of these groups is ambiguous. Ninety-five per cent of the 22 animals in experiment 3 give an ambiguous performance. In experiment 4 failure to select the anti-rachitic diet and an appreciable tendency to select the rachitic diet is evident; 30 per cent of the 27 animals chose the latter.

DISCUSSION

It is evident that the first of the simple choice experiments agrees with the finding of the maze experiments that some rats under these conditions of rachitic feeding show a persistent tendency to select the rachitic diet during a limited choice period. The discrepancies in the behavior of the different groups in the maze experiments are not explained. Two differences between the results obtained in the two types of situation may be noted. First, a larger proportion of the animals in the simple choice situation showed a decided preference for the anti-rachitic diet (68 as compared to 28 per cent of the total in the maze situation). Second, there was more variability within the separate groups in the simple choice situation. Both of these facts indicate that the maze situation contains factors which definitely modify the tendency of an animal to select the diet consistently and both suggest that factors other than nutritive condition are operative in the selections. The animals of groups II, IV, V, VI, and VII in the maze experiments had been subjected to prolonged rachitic feeding (24 to 35 days) as had the animals in experiment 4 of the simple choice experiments (26 to 30 days); and all these groups as a whole gave decidedly negative results. On the basis of the assumption that nutritive condition underlies the preference for the anti-rachitic diet, just the opposite would be expected.

To maintain that any of the individual animals tested in these experiments were selecting on the basis of nutritive condition would require that all negative cases be explained in some way that is consistent with this hypothesis. It would first be neces-

sary to assume that the 32 per cent of all animals in experiment 1 which gave negative results were not suffering from deficiency as were the rest of the same groups; or that they were unable to make the hypothetical association between nutritional condition and consumption of the better diet; or that they were unable to discriminate between the two. The first explanation is untenable, since no consistent difference was evident in the condition of positive and negative individuals, either from external observation or the weight records. The latter two explanations suppose anomalies in what well may be regarded as primitive functions biologically and seem highly improbable. In addition, on the hypotheses of nutritional need as a guiding factor in these selections, it would be necessary to explain the negative results shown by most groups in the maze experiments and by the animals in the fourth simple choice experiment. Nothing peculiar to the maze situation itself can account for the behavior shown by these negative groups, for it is not conceivable that the maze situation could inhibit the tendency to select on a nutritional basis in some animals and not in others. Again the negative results obtained in experiment 4 cannot be explained in consistency with the hypothesis in question. The delay in introduction to selection tests is the essential difference in the treatment of the animals, and this, as pointed out, should make the tendency to select the anti-rachitic diet more evident. It might be argued that the benefit obtained from the anti-rachitic diet in the brief choice period would be more limited at the extreme stage of depletion these animals had reached and that their selections were modified by this factor. This supposition, however, depends on an assumed capacity for fine adjustment between selection and beneficial effect. On the other hand, many of the rats showing positive results did so at a period when the effects of either diet must not have been very marked, and all continued to do so when the maximal proportion of anti-rachitic diet they could obtain was 20 to 30 per cent of the total food consumption. The negative cases simply cannot be made to jibe with the hypothesis of selection on the basis of nutritional need; and we are forced to fall back on some other explanation for the persistent preference for the anti-rachitic diet shown by some individuals.

What other factor may be responsible? It is evident from the control experiments (2 and 3) that this preference will not appear under certain types of maintenance feeding conditions. Animals fed on anti-rachitic diet and animals fed on stock diet did not exhibit it at all. The positive individuals, then, are not selecting on the basis of palatability independent of feeding conditions. One factor included in the feeding conditions that may possibly underlie all the preferences shown is the relative novelty of the preferred diet. Animals maintained on the rachitic diet where they showed preference at all showed it for the unfamiliar anti-rachitic diet—unfamiliar in the sense that the opportunity to consume it was limited to the brief choice period. Animals maintained on the anti-rachitic diet showed preference for the unfamiliar rachitic diet. Animals maintained on a diet entirely different from the two test foods showed hardly any preference. To explain both positive and negative results on this basis would, of course, require that we assume the tendency to select the unfamiliar diet to be subject to striking individual differences, to be extremely persistent in some animals, and to be rendered ineffective by long habituation to a particular maintenance diet. There is no evidence against these assumptions; they offer problems which must be settled by experimental test.

SUMMARY

Eleven out of 40 rats, or 28 per cent, which had selected the short path of a choice maze when rewarded by rachitic diet at the end of both paths, selected an anti-rachitic diet consistently when they were rewarded with this better diet at the end of the long path. This preference persisted in the face of a water pan obstruction and the introduction of blinds in the long path.

Sixty-eight per cent of a group of 25 rats maintained on the rachitic diet selected the anti-rachitic diet consistently in a simple choice situation with rachitic and anti-rachitic diet equally accessible.

Seventy-one per cent of a group of 17 animals maintained on the anti-rachitic diet selected the rachitic diet consistently in the same choice situation.

Ninety-five per cent of a group of 22 animals maintained on

normal stock diet showed no consistent preference for either of the test diets in the same choice situation.

None of 27 animals subjected to prolonged rachitic feeding for a period of 26 to 30 days prior to choice tests showed preference for the anti-rachitic diet in the same choice situation.

It is concluded that rats maintained on rachitic diet and permitted a brief choice period under these conditions do not select on the basis of nutritional condition. To account for decided preferences shown by a large proportion of individuals in these experiments it is concluded that the conditions of maintenance feeding involve some factor or factors responsible for such preference, and it is suggested that the factor may be the unfamiliarity of one diet relative to the other.

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